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Example 1: An Assay For Resistant Swine

The polymorphisms of the present invention are easily identified using PCR-RFLP tests. One embodiment of the tests used a 160bp fragment of porcine alpha (1,2) fucosyltransferase 1 amplified using PCR with the following primers; 5' CCAACGCCTCCGATTCTGT3' (SEQ ID NO: 1) and 5'GTGCATGGCAGGCTGGATGA3' (SEQ ID NO: 2). Preferred PCR conditions for this embodiment are 25 cycles at the following times and temperatures: 94°C, 30 sec; 60°C, 45 sec; 72°C, 90 sec. The amplified DNA from resistant swine was digested by the restriction enzyme Hgal, but was not digested by the restriction enzyme HinPI. The amplified DNA from homozygous susceptible swine was digested by the restriction enzyme HinPI. The amplified DNA from heterozygous susceptible swine was partially digested by both enzymes.

Alternatively, DNA was isolated from porcine nucleated cells according to standard procedures. Direct sequencing of porcine *FUT1* and *FUT2* sequences and their flanking regions in animals of different *ECF18R* genotype (Bb, bb) resulted in the identification of two G --> A transitions at positions 307 and 857 (termed *M307* and *M857*, respectively) of the *FUT1* ORF. The *M307* transition eliminates a restriction site for CfoI. Amplification of DNA isolated from porcine nucleated cells was preformed according to standard procedures with primers P6 and P11 (3 min at 95°C, 30 cycles of 30 sec at 95°C, 30 sec at 56°C and 30 sec at 72°C, followed by a 7 min final extension at 72°C) followed by CfoI digestion and separation on a 3% agarose gel resulted in a restriction fragment length polymorphism (RFLP). Homozygous *M307^{AA}* animals showed 2 bands. Homozygous *M307^{GG}* animals showed 93-, 241- and 87bp fragments. Heterozygous animals showed all four fragments.

25 **Example 2: Sensitivity and Specificity Of An Assay Using Alpha (1,2) Fucosyltransferase In Detecting Swine Resistant to F18 E. coli**

A study was conducted to determine the association between disease resistance and the polymorphism at position 307 of the *FUT1* gene. 183 weaned swine (ranging in ages 2-6 months) were obtained from six different breeding herds. Only one of these herds was known to contain resistant animals before the start of the study, and this herd is known to have a high incidence of porcine stress syndrome. The other 5 herds had no evidence of porcine stress syndrome, and the incidence of disease resistance was unknown. Swine from each herd were randomly selected, humanely euthanized and





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Table 1: Sequences Of Forward-(F) And Reverse-(R) Primers And Their Relative Position to the Porcine *FUT1* and *FUT2* Start Codons²

	Primer name	Primer Sequence	Position
	FUT1 P6 (R)	5'-CTTCAGCCAGGGCTCCTTAAG-3' (SEQ. ID NO:3)	+489
	FUT1 P7 (F)	5'-TTACCTCCAGCAGGCTATGGAC-3' (SEQ ID NO: 4)	+720
	FUT1 P10 (R)	5'-TCCAGAGTGGAGACAAGTCTGC-3' (SEQ ID NO: 5)	+1082
	FUT1 P11 (F)	5'-CTGCCTGAACGTCTATCAAGATC-3' (SEQ ID NO: 6)	+69
	FUT1 P16 (F)	5'-AGAGTTTCCTCATGCCACAGG-3' (SEQ ID NO: 7)	-90
10	FUT1 P18 (R)	5'-CTGCTACAGGACCACAGCATC-3' (SEQ ID NO: 8)	+1203
	FUT1 PBEST (R)	5'-ACCAAGCAGCGCAAAGTCCCTGAC GGGCACGGCCTC-3' (SEQ ID NO: 9)	+893
	FUT2 P16 (R)	5'-CTCCCTGTGCCCTGGAAGTGAT-3' (SEQ ID NO: 10)	+1094
	FUT2 P17 (F)	5'-AACTGCACTGCCAGCTTCATGC-3' (SEQ ID NO: 11)	-83

15 Table 2: Overall Recombination Fractions (θ), Lodscores (Z) And Number Of Informative Animals (N) For *M307* And Loci Of The *HAL* Linkage Group In The Landrace Experimental Population

	Locus pair	N	θ	Z
20	<i>S-ECF18R</i>	183	0.01	50.6
	<i>M307-S</i>	183	0.01	50.6
	<i>M307-ECF18R</i>	216	0.01	57.1
	<i>M307-RYR1</i>	198	0.02	47.2
	<i>M307-GP1</i>	147	0.03	34.2
25	<i>M307-PGD</i>	147	0.04	24.5

² Primers *FUT1* P10 and *FUT1* P11 are derived from the human *FUT1* gene.